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Low Accuracy of Tumor Markers for Diagnosing Pancreatic Neuroendocrine Tumors in Multiple Endocrine Neoplasia Type 1 Patients

Joanne M. de Laat, Carolina R. C. Pieterman, Maaïke Weijmans, Ad R. Hermus, Olaf M. Dekkers, Wouter W. de Herder, Anouk N. A. van der Horst-Schrivers, Madeleine L. Drent, Peter H. Bisschop, Bas Havekes, Menno R. Vriens, and Gerlof D. Valk*

Context: The assessment of tumor markers for diagnosing pancreatic neuroendocrine tumors (pNET) in multiple endocrine neoplasia type 1 (MEN1) patients is advised in the current guidelines but has never been validated for this purpose.

Objective: The objective of the study was to assess the diagnostic accuracy of chromogranin A (CgA), pancreatic polypeptide (PP), and glucagon for pNET in MEN1.

Design: This was a diagnostic study.

Setting: The study was conducted at Dutch university medical centers from 2008 to 2011, representing 90% of the total Dutch MEN1 population.

Patients and Methods: Patients for whom data on tumor markers in combination with the reference standard (ie, radiological imaging) were available between 2008 and 2011 were included. The reference standard for the presence of pNET was pathology or detection on magnetic resonance imaging, computed tomography, or endoscopic ultrasound confirmed on subsequent imaging, irrespective of modality at follow-up.

Main Outcome Measures: The area under the receiver-operating characteristic curve (AUC), positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, sensitivity, and specificity were calculated for each marker.

Results: For the analysis of PP, CgA, and glucagon, 73, 81, and 94 patients were available, respectively. The AUC for CgA was 0.48 [95% confidence interval (CI) 0.35–0.61] with a sensitivity 0.33 and a specificity 0.73; the AUC for glucagon was 0.58 (95% CI 0.46–0.70) with a sensitivity 0.43 and a specificity 0.73; and the AUC for PP was 0.64 (95% CI 0.50–0.77) with a sensitivity 0.36 and a specificity 0.74. Age, imaging modality, tumor size, and number did not influence the outcomes.

Conclusion: The diagnostic accuracy of the tumor markers CgA, PP, and glucagon for pNET in MEN1 is low. (*J Clin Endocrinol Metab* 98: 4143–4151, 2013)

Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant inherited disorder that is caused by mutations in the *MEN1* gene on chromosome 11 (1). MEN1 causes combinations of more than 20 different endocrine and nonendocrine tumors (2). The main

endocrine manifestations of MEN1 are parathyroid hyperplasia or adenomas (occurring in 78%–94% of patients with MEN1); neuroendocrine tumors (NET) of the pancreas (pNET) or duodenum (35%–75%); adrenal adenomas and adrenal nodular hyperplasia (27%–73%);

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Abbreviations: AUC, area under the ROC curve; CgA, chromogranin A; CI, confidence interval; CT, computed tomography; EUS, endoscopic ultrasound; FNA, fine-needle aspiration; LR+, positive likelihood ratio; LR–, negative likelihood ratio; MEN1, multiple endocrine neoplasia type 1; MRI, magnetic resonance imaging; NET, neuroendocrine tumor; NPV, negative predictive value; pNET, pancreatic NET; PP, pancreatic polypeptide; PPI, proton pump inhibitor; PPV, positive predictive value; ROC, receiver operating characteristic; Se, sensitivity; Sp, specificity; UMC, university medical center.

anterior pituitary tumors (20%–65%); and gastric, thymic, or lung NETs (8%) (3, 4). Signs and symptoms are a consequence of hormone overproduction, local mass effects, or malignancy (5). Most MEN1-associated tumors are benign, but pancreatic and thymic NETs especially show malignant behavior. Therefore, MEN1 patients have a shorter life expectancy than the general population with a 20-year survival of patients affected with MEN1 of 64% (6). However, among two families in northern Finland with a founder *MEN1* mutation, the mean age of death for MEN1-affected patients and their spouses did not significantly differ (7).

Today MEN1-related mortality is most frequently caused by pNET and thymic NET (8). For pNET, screening and subsequent treatment in asymptomatic MEN1 patients seems to lead to a more favorable survival and a decreased morbidity (5, 9).

The current standard of care for MEN1 patients is based on the recently updated clinical practice guidelines for MEN1 (2, 10). The biochemical tests recommended for early diagnosing of pNET include the annual assessment of chromogranin A (CgA), pancreatic polypeptide (PP), and glucagon.

Data on the diagnostic accuracy of the tumor markers for diagnosing pNET in MEN1 patients is scarce. Elevated levels of CgA seemed to be specific for identifying neuroendocrine tumors when patients without MEN1 were compared with healthy controls, but sensitivity was variable (11, 12). Some studies have suggested that levels of CgA might be elevated in MEN1 patients in the absence of active disease (13–15).

To achieve an effective screening program for *MEN1* carriers, more information is needed about the diagnostic value of these biochemical tests. Therefore, the aim of the present study was to assess the accuracy of the already often used CgA, glucagon, and PP for diagnosing pNET in the periodic follow-up of MEN1 patients.

Materials and Methods

The present study was based on data from the national MEN1 database of the DutchMEN1 Study Group (DMSG). The database comprises 90% of the total Dutch MEN1 population (16) of whom the available clinical data of every quarter per year was collected for the period 1990 up to 2011. Data collection was performed for every quarter using a predefined protocol and decision rules for registration of data. The database was designed to assess the effec-

tiveness of the current practice of care for patients with MEN1. In each center, MEN1 patients were identified by a standard identification procedure using the hospital databases of medical conditions and diseases. All patients with MEN1 aged 16 years or older by the end of 2010 who were treated at one of the university medical centers (UMCs) were included. MEN1 was diagnosed according to the recently updated clinical practice guidelines (2). The study protocol was approved by the Medical Ethical Committees of all UMCs in The Netherlands. Given the retrospective and observational nature of the study, the Medical Ethical Committees approved the use of these clinical data for study aims and waived the requirement to obtain informed consent. The data were collected after the study questions were formulated and the potential variables of interest were identified.

Patient selection

In the present analyses, patients under care between 2008 and 2011 ($n = 274$) were eligible. This period was chosen to be able to study the currently used and most up-to-date biochemical and radiological tests.

Patients were included in the analyses if the presence or absence of pNET could be assessed according to the reference standard described below. Patients were excluded from the analyses if laboratory results of interest were not measured within 3 months before or after assessing the reference standard.

Increased levels of CgA can also be caused by other NETs (17, 18). For this reason additional selection criteria were applied in the analysis of CgA. First, patients with simultaneous thymic, lung, or gastric NET were excluded. Because patients with gastrinomas, and especially those with Zollinger Ellison syndrome, are currently treated with proton pump inhibitor (PPI), we performed an additional subgroup analysis for PPI use in the analysis of CgA.

Patients with missing data were excluded per analysis.

Outcome measures

The reference standard for the presence of pNET was the outcome of a pathology examination. If pathology was not available, only pNET diagnosed on magnetic resonance imaging (MRI), computed tomography (CT), or endoscopic ultrasound (EUS), which were confirmed at least once by consecutive imaging studies, irrespective of imaging modality, were considered as pNET positive. The absence of pNET also had to be confirmed on a minimum of two subsequent imaging studies during follow-up.

Primary outcomes were the accuracy of PP, glucagon, and CgA for diagnosing pNET according to the reference standard.

Secondary outcomes were the diagnostic accuracy of the combined tumor makers for pNET and diagnostic accuracy for metastatic disease. The reference standard for metastatic disease was defined as metastases confirmed in pathological examination (metastases in liver, lymph nodes, and peritoneum) or metastases identified on abdominal MRI, CT, or EUS examination (metastases in liver, lymph nodes, bones, peritoneum).

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Additionally, we assessed the association of tumor markers with age, tumor size, and number of tumors. Furthermore, we analyzed the diagnostic value for the different imaging modalities and the available data on octreoscans and EUS with fine-needle aspiration (FNA). Because of the interaction between PPIs and CgA, all analyses of CgA were categorized for PPI use (19).

For the analysis we used the standard upper limits of reference values for the tumor markers as currently used in clinical practice. Although previous studies have reported some age-dependent regression for PP, generally no correction for this regression is performed in daily clinical practice (20). Additionally, to assess whether a significant influence of age on the results exists, we performed a sensitivity analysis using age-adjusted reference values for tumor markers that had a significant correlation with age.

Tumor markers

All laboratory results analyzed were obtained from tumor marker assessments performed in the course of patient care between 2008 and 2011. Tumor marker assessments were performed centralized in a small number of laboratories. PP analysis was performed in one center using a RIA from Euro-Diagnostica. Long-term assay stability was measured using patients pools at three levels, viz, at 27, 92, and 170 pmol/L. These patient pools were used from 2008 until 2011. Three antibody kits were used between 2008 and 2011. Interassay variation was 10%, 6%, and 6% for these three kits. Cross-reactivity for insulin, glucagon, gastrin-34, and gastrin-17 is less than 0.03%.

The CgA assays were performed in two centers both using the Cis Bio immunoradiometric assay with identical reference values. Interassay variation was 9%, 10%, and 6% at 34, 72, and 271 $\mu\text{g/L}$. Both laboratories used patient pools at three levels for the long-term control during the study period. Each batch of pools was used for about 1 year. Five lot numbers of reagents for CgA analysis were used.

Glucagon assays were performed in four laboratories using a RIA from Siemens Healthcare Diagnostics or Linco Diagnostics. It is standardized against World Health Organization International Standard 69/194 (21). Interassay variation was 12%, 12%, and 7% at 16, 23, and 40 pmol/L. Centers did not use patient pools during the whole study period. However, all laboratories report that the overall interassay coefficient of variation was less than 10% over the time a batch of control samples was used. No systematic decrease or increase was seen in that time. Cross-reactivity for glucagon 22–29 is 5%–10% and for glucagon 19–29 it is 2.3–4.7% (information from manufacturer). PPs are represented in picomoles per liter and CgA tests in micrograms per liter. The laboratories of the UMCs reported the levels of glucagon in different units, respectively, nanograms per liter and picomoles per liter. For the analyses, the measure picomoles per liter was converted to nanograms per liter (1 ng/L = 0.2872 pmol/L). All four laboratories used identical reference values for glucagon, and therefore, no standardization of tumor marker values was needed.

Currently used upper limits of reference values are for PP 100 pmol/L, for glucagon 80 ng/L, and for CgA 100 $\mu\text{g/L}$ as provided by the manufacturer of the diagnostic kits and based on previous studies (12, 14, 17, 20).

Statistical analysis

Diagnostic accuracy was assessed using receiver operating characteristics (ROC) curves. The area under the ROC curves

(AUC) were calculated with an AUC of 0.60–0.80 indicating moderate, and 0.80–1.00 indicating good diagnostic accuracy. The current reference values of the tumor markers were used as cutoff for the three tumor markers. For the analysis of the combined tumor markers, the tumor markers were considered positive if one or more tumor makers were elevated. We also intended to calculate the optimal cutoff using the ROC curves for tumor markers with an AUC above 0.8.

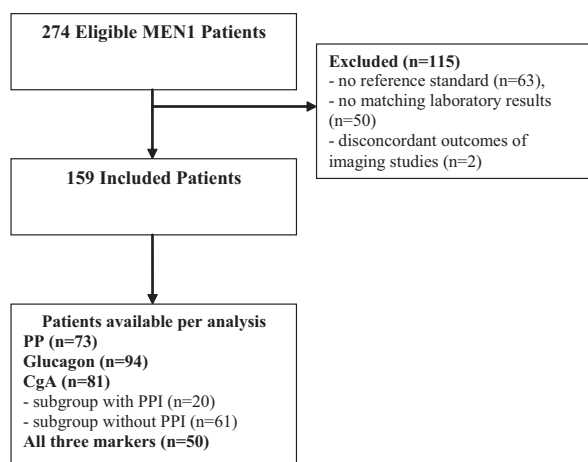
Diagnostic accuracy with age-adjusted reference values was obtained first by calculating the difference between the observed tumor marker value and the predicted tumor marker value. Second, the AUC was calculated for sensitivity (Se) and specificity (Sp) using the following formulas: $\text{Se} = \text{true positive}/(\text{true positive} + \text{false negative})$ and $\text{Sp} = \text{true negative}/(\text{true negative} + \text{false positive})$. The positive (PPV) and negative predictive value (NPV) were calculated using the following formulas: $\text{PPV} = \text{true positive}/(\text{true positive} + \text{false positive})$; $\text{NPV} = \text{true negative}/(\text{true negative} + \text{false negative})$. The positive (LR+) and negative (LR–) likelihood ratios were calculated using the following formulae: $\text{LR+} = \text{sensitivity}/(1 - \text{specificity})$, $\text{LR-} = (1 - \text{sensitivity})/\text{specificity}$. In addition, exact 95% confidence intervals (CIs) of the predictive values and likelihood ratios were calculated according to the methods described by Altman (22). Scatterplots were made to assess the observed results of tumor marker levels for individual patients. The association of tumor makers with age, tumor size, and number of tumors was assessed by subgroup analyses and by linear regression. For subgroup analyses, categorizations of data for pNET size, pNET number, and age were based on the median (data < or \geq median). Linear regression was applied to logistically transformed data of tumor makers, age, tumor size, and number of tumors to diminish the impact of extreme values. Regression coefficients with $P < .05$ were considered significant.

To assess whether age-adjusted reference values would influence study results, we performed a sensitivity analysis based on age-adjusted reference values. Reference values were internally adjusted for age using linear regression by calculating the difference between the standard reference value and the calculated age adjusted tumor marker value according to the outcome of the regression analyses of tumor markers and age. In addition, types of imaging test (CT, MRI, or EUS) were separately analyzed, and for CgA, a stratified analysis for PPI use was performed. Analyses were conducted using SPSS 17.0 and R version 2.9.2.

Results

Study population

Of the total of 274 eligible MEN1 patients, 159 patients were included (Figure 1). Seventy-three, 81, and 94 of those 159 patients were available for analysis of PP, CgA, and glucagon, respectively. In 50 patients all three tumor markers were assessed. Median age of the patients at the baseline assessment was 44 years (range 16–78 years). There were more females than males (94 females and 65 males). Age and gender of the excluded and included patients were not statistically different.



CgA: chromogranin A
PP: pancreatic polypeptide
NET: neuroendocrine tumor
PPI: proton pump inhibitors
n: number of patients

Figure 1. Flow chart of patient enrolled in the study for diagnostic accuracy of tumor markers for pNET.

Tumor markers for diagnosing pNET

The AUCs of CgA, PP, and glucagon were small for all tumor markers. For patients not using PPI, the AUC of CgA was also low [0.56 (95% CI 0.41–0.70)]. The outcomes of the Se, Sp, LR+, LR–, and AUCs are listed in Table 1. The diagnostic accuracy was comparable when an optimal cutoff point for these data was used.

Figure 2 represents the AUCs and the scatterplots of the outcomes of the tumor markers in individual patients with and without pNET according to the reference standard.

The analysis of the combined tumor marker CgA, PP, and glucagon was based on all 159 patients. The AUC was 0.59 (95% CI 0.50–0.68) with a LR+ of 1.50 (95% CI 1.01–2.22) and a LR– of 0.74 (95% CI 0.59–0.92). A total of 27 patients had elevated tumor markers in the absence of pNET on imaging. In those patients the mean time between first and last imaging was 2 years and a mean number of 3.2 additional imaging studies was performed per patient, confirming the negative outcome of the reference standard.

Tumor markers for metastatic disease

The Se, Sp, LR+, LR–, and AUCs of the individual tumor markers for diagnosing metastatic disease are presented in Table 2. The prevalence of metastatic disease in the population ranged from 7% to 12% in separate analyses, depending on the number of patients included. The LR+ ranged from 1.50 to 1.89 and LR– from 0.66 to 0.75.

Factors influencing diagnostic accuracy

Correlations between the level of the tumor markers and tumor size, number, or age were weak, with R² ranging between less than 0.001 and 0.061 (Figure 3). The only

Table 1. Accuracy of the Tumor Markers for Diagnosis of pNET

Tumor Marker	Outcome of Reference Standard (n)			Se ^a and Sp ^a	PPV ^a and NPV ^a	LR+ ^a and LR– ^a	AUC ^a
	Positive	Negative	Total				
CgA, $\mu\text{g/L}^b$							
Positive	18	7	25	Se 0.33 (0.21–0.47)	PPV 0.72 (0.51–0.88)	LR+ 1.22 (0.58–2.54)	0.48 (0.35–0.61)
Negative	37	19	56	Sp 0.73 (0.52–0.88)	NPV 0.34 (0.22–0.48)	LR– 0.92 (0.74–1.14)	
Total	55	26	81				
CgA without PPI, $\mu\text{g/L}^b$							
Positive	9	1	10	Se 0.21 (0.11–0.37)	n.d.	LR+ 4.07 (0.55–29.9)	0.56 (0.41–0.70)
Negative	33	18	51	Sp 0.95 (0.72–1.00)		LR– 0.83 (0.70–0.98)	
Total	42	19	61				
CgA with PPI, $\mu\text{g/L}^b$							
Positive	9	6	15	Se 0.69 (0.39–0.90)	n.d.	LR+ 0.81 (0.51–1.29)	0.47 (0.22–0.73)
Negative	4	1	5	Sp 0.14 (0.01–0.58)		LR– 2.15 (0.18–26.1)	
Total	13	7	20				
Glucagon, ng/L							
Positive	26	9	35	Se 0.43 (0.30–0.56)	PPV 0.74 (0.57–0.88)	LR+ 1.56 (0.83–2.93)	0.58 (0.46–0.70)
Negative	35	24	59	Sp 0.73 (0.54–0.87)	NPV 0.41 (0.28–0.54)	LR– 0.79 (0.62–1.00)	
Total	61	33	94				
PP, pmol/L							
Positive	18	6	24	Se 0.36 (0.23–0.51)	PPV 0.75 (0.53–0.90)	LR+ 1.38 (0.63–3.01)	0.64 (0.50–0.77)
Negative	32	17	49	Sp 0.74 (0.52–0.90)	NPV 0.35 (0.22–0.50)	LR– 0.87 (0.68–1.10)	
Total	50	23	73				

Abbreviation: n.d., not determined.

^a With 95% CI.

^b In the CgA analyses patients with lung, thymic, and gastric NET were excluded.

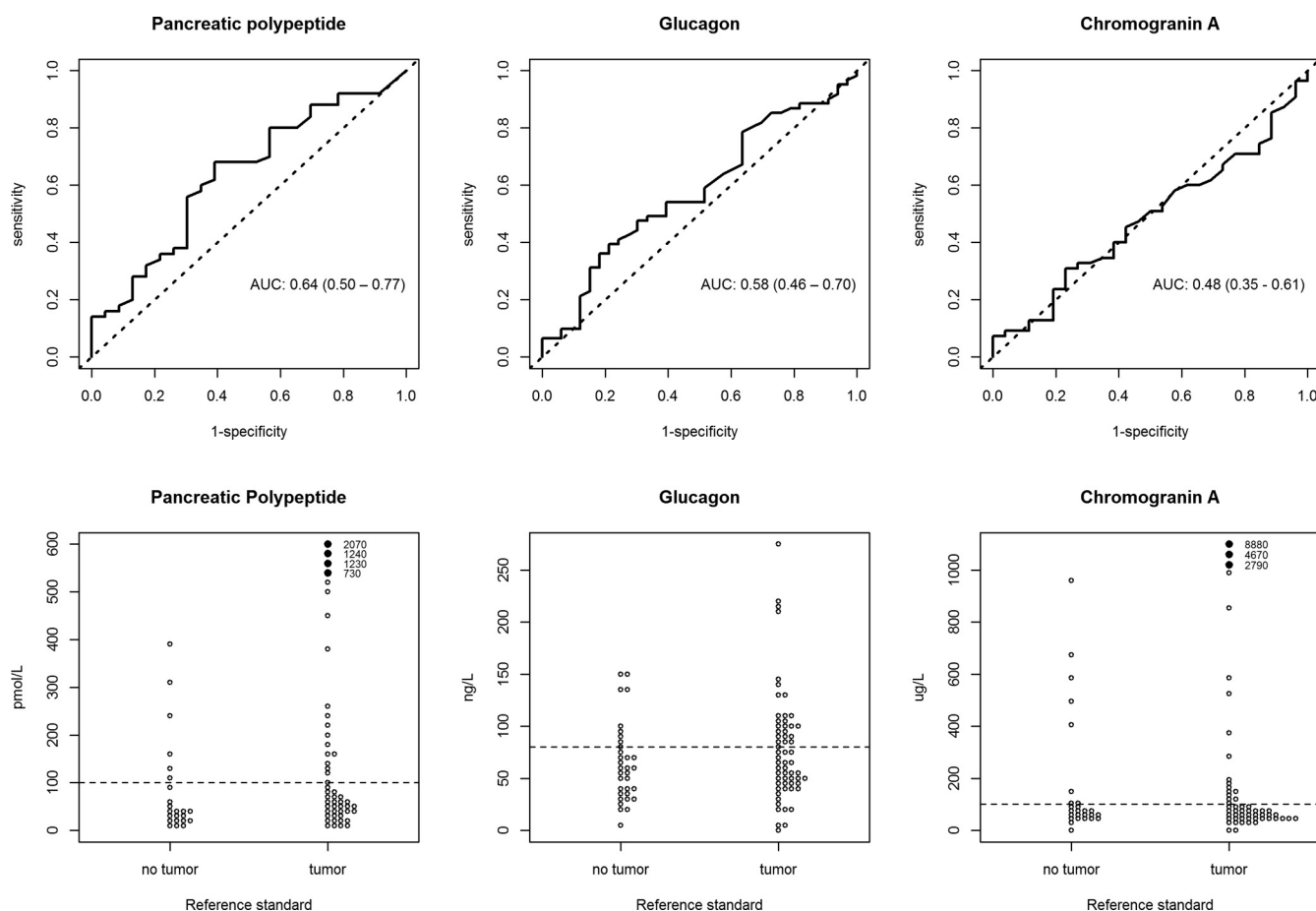


Figure 2. ROC curves of tumor markers for pNET with scatter plots of the observed results of tumor marker levels for individual patients with and without pNET.

significant correlation found was between PP and age ($P = .036$). Adjusting for age did not improve diagnostic accuracy of PP (AUC 0.57 95% CI 0.43–0.71). An additional categorized analysis also showed no significant difference in the AUC of tumor markers with tumor size, tumor number, or age (Table 3).

The accuracy of the tumor markers was not influenced by type of imaging used (CT, MRI, or EUS) (Table 4). There were no statistically significant differences in the diagnostic accuracy of CgA in stratified analyses for PPI use.

Octreoscans were performed in 59 patients and were often performed to exclude metastatic disease in patients

Table 2. Accuracy of the Tumor Markers for Metastatic Disease

Tumor Marker	Outcome of Reference Standard (n)			Se ^a and Sp ^a	PPV ^a and NPV ^a	LR+ ^a and LR– ^a	AUC ^a
	Positive	Negative	Total				
CgA, $\mu\text{g/L}^b$							
Positive	8	31	39	Se 0.53 (0.27–0.79)	PPV 0.21 (0.09–0.36)	LR+ 1.82 (1.04–3.19)	0.66 (0.49–0.83)
Negative	7	75	82	Sp 0.71 (0.61–0.79)	NPV 0.91 (0.83–0.96)	LR– 0.66 (0.38–1.14)	
Total	15	106	121				
Glucagon, ng/L							
Positive	4	36	40	Se 0.50 (0.16–0.84)	PPV 0.10 (0.03–0.24)	LR+ 1.50 (0.71–3.15)	0.74 (0.60–0.88)
Negative	4	72	76	Sp 0.67 (0.57–0.75)	NPV 0.95 (0.87–0.99)	LR– 0.75 (0.37–1.51)	
Total	8	108	116				
PP, pmol/L							
Positive	4	19	23	Se 0.50 (0.16–0.84)	PPV 0.17 (0.05–0.39)	LR+ 1.89 (0.86–4.19)	0.73 (0.54–0.92)
Negative	4	53	57	Sp 0.74 (0.62–0.83)	NPV 0.93 (0.83–0.98)	LR– 0.68 (0.33–1.37)	
Total	8	72	80				

Abbreviation: n.d., not determined.

^a With 95% CI.

^b In the CgA analyses patients with lung, thymic, and gastric NET were excluded.

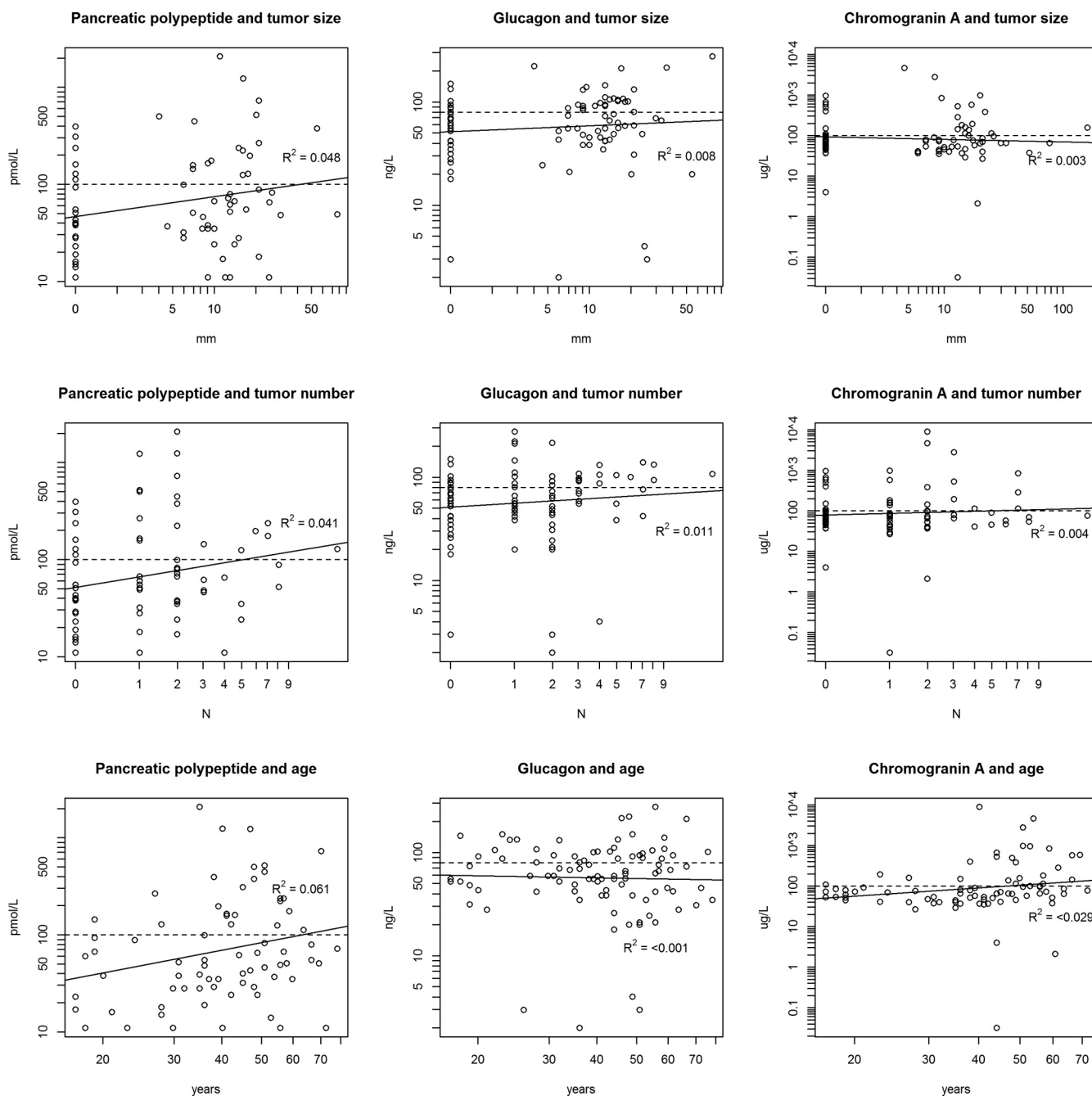


Figure 3. Scatter plots with fitted regression lines showing the correlation between tumor marker levels and tumor size, tumor number, and age.

previously diagnosed with pNET (2). Octreoscans showed pancreatic uptake in 23 patients, and 21 had previously been diagnosed with pNET on conventional imaging. PP and glucagon had greater accuracy for pNETs positive on octreoscan, PP (AUC 0.79; 95% CI 0.63–0.96), and glucagon (AUC 0.81; 95% CI 0.63–0.98). There was no difference in the accuracy of CgA for lesions positive on octreoscans compared with other modalities of imaging.

In 51 patients pNET was diagnosed using EUS. EUS was followed by FNA in 9 cases. FNA was positive for pNET in seven patients. In the other two patients, samples obtained by FNA were not representative.

Discussion

In this study the accuracy of CgA, PP, and glucagon for diagnosing pNET in MEN1 patients in daily clinical practice was low. In clinical practice, tumor markers for diagnosing pNET are often measured simultaneously. Our results showed, that even if the three tumor markers are measured in combination, the diagnostic accuracy can be judged to be insufficient for diagnostic use. In a stratified analysis, the tumor markers did not perform better in subgroups based on age, tumor size, number of pNETs, and type of imaging. For the assessment of metastatic disease,

Table 3. Accuracy of the Tumor Markers for Diagnosing pNET Categorized for Tumor Size, Tumor Number, and Age

Tumor Marker	AUC Tumor Size < Median ^a	AUC Tumor Size ≥ Median ^a	AUC Tumor Number < Median ^a	AUC Tumor Number ≥ Median ^a	AUC Age < Median ^a	AUC Age ≥ Median ^a
CgA, $\mu\text{g/L}$ ^b	0.42 (0.27–0.58)	0.51 (0.35–0.67)	0.44 (0.27–0.61)	0.51 (0.36–0.66)	0.41 (0.23–0.58)	0.51 (0.30–0.74)
Glucagon, ng/L	0.56 (0.42–0.70)	0.60 (0.45–0.75)	0.60 (0.44–0.76)	0.57 (0.44–0.70)	0.64 (0.48–0.81)	0.54 (0.36–0.72)
PP, pmol/L	0.57 (0.41–0.73)	0.70 (0.54–0.86)	0.58 (0.39–0.76)	0.67 (0.52–0.82)	0.64 (0.46–0.82)	0.61 (0.39–0.83)

Median tumor size was 13 mm; median tumor number was 2; and the median age was 44 years.

^a With 95% CI.

^b In the CgA analyses patients with lung, thymic, and gastric NET were excluded.

AUCs were slightly higher. PP and glucagon showed higher diagnostic accuracy for pNETs positive on octreoscans; however, these results should be interpreted cautiously because octreoscans were performed in a selected group of patients who had already been diagnosed with pNET previously.

Strengths and limitations

To our knowledge, this study is the first to assess the diagnostic accuracy of tumor marker for pNET in a large cohort of MEN1 patients. For the DMSG database, we collected the data of 90% of the total MEN1 patient population, and this database is therefore a true population-based database, reducing the chance of selection bias.

The prevalence of pNET in our study population was comparable with that in other MEN1 cohorts; thus, our results seem generalizable (23, 24). Results on the diagnostic accuracy of tumor markers were also consistent in multiple stratified analyses, suggesting a low risk of bias.

A few limitations should be discussed. First, pathology is the preferred reference standard but often not available. In patients lacking pathology, we used the results of MRI, CT, and EUS as the reference standard. The use of multiple reference tests might overestimate the diagnostic accuracy of tumor makers (25). Diagnostic accuracy might further be overestimated because the imaging studies and tumor marker assessments were performed in the course of patient care and were not blinded or standardized. To reduce the

impact of these limitations, we required positive or negative imaging tests to be confirmed by at least one consecutive test in the course of follow-up. In this manner, the consistency of imaging results was part of the reference standard, thereby reducing the chance of false-negative or false-positive findings. Furthermore, we used imaging results only from 2008 onward because the accuracy of radiological and EUS examinations have improved in recent years, with a sensitivity of 93% and a specificity up to 95% (26). Importantly, despite these limitations that can lead to overestimating diagnostic accuracy, the diagnostic value of all three markers was low in all analyses.

Another limitation is that numbers, especially for subgroup analyses, were rather small, leading to imprecise estimates of diagnostic test performance. This imprecision is captured in the limits of the CI. To circumvent the power problem inherent to binary data, we also performed an analysis based on linear models, regressing tumor marker levels and predictive values such as age and tumor size (after logarithmic transformation).

The diagnostic value of the three combined tumor markers might have been underestimated in our assessment because only one or two tumor markers were assessed in many patients.

The use of standard reference values as currently used in daily clinical practice instead of age-adjusted reference values seems justified because diagnostic accuracy did not

Table 4. Accuracy of the Tumor Markers for Diagnosis of pNET Categorized for Modality of Imaging

Tumor Marker	CT AUC ^a	MRI AUC ^a	EUS AUC ^a
CgA, $\mu\text{g/L}$ ^b	0.54 (0.39–0.68) (n = 64)	0.44 (0.30–0.57) (n = 70)	0.40 (0.19–0.61) (n = 45)
Glucagon, ng/L	0.56 (0.39–0.73) (n = 54)	0.65 (0.52–0.77) (n = 82)	0.57 (0.27–0.88) (n = 40)
PP, pmol/L	0.64 (0.49–0.80) (n = 47)	0.56 (0.39–0.73) (n = 51)	0.53 (0.29–0.77) (n = 41)

Abbreviation: n, number of patients.

^a With 95% CI.

^b In the CgA analyses patients with lung, thymic, and gastric NET were excluded.

improve in the sensitivity analyses using age-adjusted reference values.

Biochemical testing of gastrin, vasoactive intestinal peptide, glucose, insulin, and C-peptide has also been suggested in the diagnosis of pNET (2). We did not assess the diagnostic accuracy of these tests for the following reasons: fasting glucose, C-peptide, and insulin were assessed when insulinoma was suspected based on clinical symptoms, and high levels of gastrin can also originate from submucosal duodenal gastrinomas, which cannot be detected reliably on conventional imaging such as MRI and CT scans. Vasoactive intestinal peptide seldom was assessed in our population.

Comparison with other literature

In previous studies elevated levels of CgA were specific for identifying neuroendocrine tumors when non-MEN1 patients were compared with healthy controls, but sensitivity was variable (11, 12, 26, 27). A few small studies already suggested that sensitivity and specificity might be lower in MEN1 patients (14, 15). However, in these studies there was a possible selection bias, and criteria for diagnosing pNET were often not clearly described. Our findings in a relatively large and less biased patient sample now show that the AUCs of the tumor markers for diagnosing pNET in MEN1 patients were low. The accuracy of tumor markers for diagnosing patients with metastatic disease was slightly higher but still low. These findings are in line with a recent study of 115 patient with gastroenteropancreatic NET, in which the tumor markers CgA and PP were of limited value in diagnosing metastatic disease (28). In this study, only seven MEN1 patients were included.

Clinical implications

Tumor makers are of low diagnostic value for detecting pNET in MEN1 patients. Therefore, we think that imaging should be the preferred method of screening for pNET. In times of evidence-based and cost-effective care, it is questionable whether tumor markers must be used for the periodic assessment of MEN1 patients for diagnosing pNET. The annual costs of the measurements of the tumor markers CgA, PP, and glucagon are estimated at approximately € 110 per patient, according to the 2004 price list of the Dutch Health Care Insurance Board. The costs of imaging are approximately €130 for CT and €180 for MRI, leading to annual costs of €65–€90 when these are performed once per 2 years, respectively. However, one study suggested that once a tumor is identified, these biochemical markers, if elevated, can possibly be useful in the follow-up of the treatment effect (29).

Conclusions

The value of CgA, PP, and glucagon for diagnosing pNET in MEN1 patients is low. Longitudinal studies are now needed to examine the value of CgA, glucagon, and PP as markers in the follow-up of MEN1 patients with pNET.

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